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8/a B. Webb 2/7/01

Attorney Docket No. P50572X1

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	DeBouck, *et al.*	24 January 2001
Serial No.:	09/297,701	Group Art Unit No.: 1655
Filed:	May 5, 1999	Examiner: Souaya, J.
For:	METHODS FOR IDENTIFYING GENES ESSENTIAL TO THE GROWTH OF AN ORGANISM	

Assistant Commissioner of Patents
Washington, D.C. 20231

PRELIMINARY AMENDMENT

Sir:

Preliminary to calculating filing fees and examining this application, please amend the application as follows:

IN THE CLAIMS:

Please amend claims 1, 3, 4, 10, 11, and 12 as follows:

1. (Amended) A method of identifying genes essential to growth of a single celled organism[selected organism] comprising:

(a) preparing a genomic library of the single celled organism[a selected organism];

(b) providing a plurality of identical grids, each grid comprising a surface on which is immobilized at predefined regions on said surface a plurality of defined materials derived from the genomic library;

(c) mutagenizing the single celled organism by transfection with *(i)* a randomly integrated transposon or *(ii)* a similar insertional or transposable element of known sequence or *(iii)* with a constructed suicide vector[selected organism];

(d) growing a test culture comprising the mutagenized single celled organisms[selected organism] and a control culture comprising non-mutagenized single celled organisms[selected organism] under a set of defined conditions;

(e) harvesting surviving cells from the cultures;

(f) extracting and isolating DNA from harvested cells of the test culture;

(g) extracting and isolating RNA or DNA from harvested cells of the control culture;

(h) generating labeled polynucleotide probes from the isolated DNA of the test culture;

(i) generating labeled polynucleotide probes from the isolated RNA or DNA of the control culture;

(j) hybridizing the labeled probes generated from the isolated DNA of the test culture to a first identical grid to produce a test hybridization pattern;

(k) hybridizing the labeled probes generated from the isolated RNA or DNA of the control culture to a second identical grid to produce a control hybridization pattern;

(l) comparing the hybridization patterns to identify genes essential for growth in the single celled organism[of the selected organism]; and

(m) confirming that said identified gene is essential for growth of the single celled organism.[selected organism.]

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3. (Amended) The method of claim 1 wherein the set of defined conditions of step (d) comprises standard non-pathogenic *in vitro* culture conditions for the single celled organism.[selected organism.]

4. (Amended) The method of claim 1 wherein the set of defined conditions of step (d) comprises *in vitro* conditions which reflect or mimic *in vivo*, pathogenic settings including[such as] aerobic or anaerobic conditions, auxotrophic, heat-shock, osmotic-shock, addition or presence of antibiotics or drugs, carbon source variations, and *in vivo* pathogenic conditions.

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10. (Amended) The method of claim 9 wherein genes essential to the single celled organism[selected organism] are identified by determining identical hybridization patterns for all of the cells grown under the different sets of defined conditions.

11. (Amended) The method of claim 9 wherein genes essential to the single celled organism[selected organism] are identified by determining differences between the test and control hybridization patterns for cells grown under the different sets of defined conditions.

12. (Amended) A method of identifying genes essential to growth of a single celled organism[selected organism] by identifying conditionally lethal mutant genes, which comprises:

(a) preparing a genomic library of the single celled organism:[a selected organism:] (*i*) in an integration vector; or (*ii*) in an expression vector;

(b) providing a grid comprising a surface on which is immobilized at predefined regions on said surface a plurality of defined materials derived from the genomic library;

(c) mutagenizing the single celled organism:[selected organism:] by transfection with *(i)* a randomly integrated transposon or *(ii)* a similar insertional or transposable element of known sequence or *(iii)* with a constructed suicide vector;

(d) growing the mutagenized single celled organisms[organism] under permissive and non-permissive conditions to identify mutagenized single celled organisms[organisms] containing conditionally lethal mutant genes;

(e) transforming the single celled organism[such organisms] containing said conditionally lethal mutant genes with the genomic library of step (a);

(f) growing the transformed cells under the same non-permissive conditions as step (d) to identify transformed cells in which conditionally lethal mutant genes have been complemented;

(g) harvesting surviving cells;

(h) extracting and isolating DNA from the harvested cells;

(i) generating labeled polynucleotide probes from the isolated DNA;

(j) hybridizing the labeled probes generated from the isolated DNA to a grid, whereby such probes that hybridize to the grid identify genes essential for growth of the single celled organism.[selected organism.]

REMARKS

The amendments to claims 1, 3, 4, 10, 11, and 12 are merely editorial in nature. Support for the amendment "single celled organism" can be found in the specification as originally filed at page 5, lines 19-22. Support for the amendments to claim 1(c) and 12(c) can be found in the

specification as originally filed at pages 9-13. As none of these amendments introduce new matter, entry thereof is believed to be in order, and is respectfully requested.

If it would expedite the prosecution of this application, the Examiner is invited to confer with the Applicants' undersigned attorney.

Respectfully submitted,

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